



TITLE:

Studies on the Metabolic Products of *Pseudomonas aeruginosa* sp. (II) On the Quantitative Determination of Pyocyanine. (1)

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CITATION:

Kurachi, Mamoru. Studies on the Metabolic Products of *Pseudomonas aeruginosa* sp. (II) On the Quantitative Determination of Pyocyanine. (1). Bulletin of the Institute for Chemical Research, Kyoto University 1954, 32(2): 105-106

ISSUE DATE:

1954-03-31

URL:

<http://hdl.handle.net/2433/75413>

RIGHT:

The results demonstrate that this purified pyrophosphatase might differ from the ATP-ase. But it is not clear whether the enzyme which hydrolyses co-carboxylase is identical with the purified pyrophosphatase or not.

22. Studies on the Metabolic Products of *Pseudomonas aeruginosa* sp. (II)

On the Quantitative Determination of Pyocyanine. (1)

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Since any report on the quantitative determination of pyocyanine had not yet been put forward, in the previous investigation amounts of pyocyanine were compared merely by colorimetric observation.

In the present paper, experimental results on photometric determination of pyocyanine by using BECKMAN model DU quartz spectrophotometer are mainly dealt with.

After various attempts, the crystal of blue pigment obtained from cultural solutions of the bacteria was ascertained to be identical with pyocyanine itself by elementary analysis. It was observed that pure crystal was never obtained by repeating recrystallization, whereas blue crystals were easily obtained from concentrated chloroform solution of pyocyanine by adding ethyl ether and then recrystallized from water as very fine needles.

On the other hand, crystals of pyocyanine were successfully obtained from its chloroplatinate ($(C_{13}H_{10}N_2O)_2H_2PtCl_6$). From the fact mentioned above, it will be suggested that not only destruction of pyocyanine takes place during purification, but its crystal is also contaminated by impurities revealing similar solubility to the solvents.

Besides pyocyanine chloroplatinate, various salts of inorganic acids were obtained. It is noteworthy that with many kinds of organic acids except oxalic acid, pyocyanine salt could not be crystallized. With regard to the absorption spectrum of pyocyanine, several reports have hitherto been put forward, but these results contradicted each other. For the determination of pyocyanine, the author reexamined its absorption spectrum and obtained the following results differed considerably from those of other investigators: with neutral or alkaline pyocyanine solution, absorption maxima are 690 $m\mu$, 379 $m\mu$, 311 $m\mu$ and 238 $m\mu$, and minima are 448 $m\mu$, 344 $m\mu$ and 270 $m\mu$; with acid solution, maxima are 520 $m\mu$, 387 $m\mu$, 278 $m\mu$, and 242 $m\mu$, and minima are 422 $m\mu$, 318 $m\mu$, 251 $m\mu$ and 231 $m\mu$.

Among several reports, the results by Ehrismann et al. (*Biochem. Z.*, 284, 476 (1936)) coincide to some extent with the present results in the form of absorption curves, though all the absorption bands except at 520 $m\mu$ do not coincide with those of the author. On the other hand, α -oxyphenazine ($C_{12}H_8N_2O$) is found to be an impurity accompanied by pyocyanine. Therefore it is significant to examine the absorption spectrum of α -oxyphenazine in order to test its effect on the determination of pyocyanine.

It is found that α -oxyphenazine shows a slight absorption band at 51 $m\mu$ in visible region only when its solution is kept alkaline. Accordingly, the effect of the contamination of α -oxyphenazine on the photometric determination can be concluded to be negligible. Pyocyanine chloroplatinate was observed to be rather easily purified and it was considerably stable, so that chloroplatinate was used for the estimation of molecular extinction coefficient (ϵ) of pyocyanine ($C_{13}H_{10}N_2O$:210.09) and found to be as follows:

$\epsilon=4300$ at 690 $m\mu$ with pyocyanine aqueous solution, or $\epsilon=2400$ at 520 $m\mu$ with pyocyanine-HCl aqueous solution.

Using this coefficient, amount of pyocyanine in the medium mentioned in the previous paper (This Bulletin, 25, 71 (1951)) was calculated as 0.2835 g. per liter. This amount attains to about thirty times to the yield reported by other authors (Fr. Wrede u. E. Strack: *Z. f. physiol. Chem.*, 181 59 (1929)).

23. Studies on the *Propionibacterium*. (V)

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Researches on the mechanism of propionic acid fermentation by *Propionibacterium* were hitherto undertaken on anaerobic conditions, and the chemical changes in the fermentation are suggested as follows: 1) pyruvic acid is produced from sugars by EMBDEN-MEYERHOF-PARNAS schema; 2) pyruvic acid is converted into acetic acid by oxidative decarboxylation; 3) succinic acid is formed by successive reduction after fixation of CO_2 to pyruvic acid; 4) propionic acid accumulates by decarboxylation of succinic acid.

The fermentation under aerobic condition (shaking culture) was carried out in the present paper and found that the products differed from those of anaerobic condition (under reduced pressure) as will be seen in Table 1; 1) crop yield of the bacteria was increased and the total acids produced in the medium was decreased; 2) greater amount of acetic acid, but less amount of propionic acid were formed, although succinic acid was always recognized in each case.